Bulletin No. 161. June, 1930

Georgia Experiment Station
EXPERIMENT, GEORGIA

"Halo Spot" of Beans and Kudzu

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EXPERIMENT, GEORGIA

"HALO SPOT" OF BEANS AND KUDZU

A bacterial (*Bacterium medicaginis* var. *phaseolicola* Burk.) disease of beans, now generally known as "Halo Spot"[5] was described by Burkholder[1] as occurring in New York State during 1926. Since that time it has been found in most bean-growing sections of the United States[3], [4], [5], [7] and has also been reported from Europe[2], [6]. It was reported from southern Georgia in 1928 but was not found on beans in the Piedmont section of the State until 1929.

A bacterial "Halo Spot" of kudzu was described by Miss Hedges[4] 1928 and attributed to a new species *Bacterium puerariae*. Recently the same author[6] has found that this organism is identical with that causing the bean disease and accepts the name applied previously by Burkholder.

During the first half of 1929 rains were frequent and the air was constantly humid, conditions which appear to be necessary for rapid spread of this disease which was first noticed on May 18th.

In passing the string-bean variety plats of the Experiment Station, a marked yellowing and stunting of certain varieties was noticeable at some distance from the field. Upon closer examination this was found to be due to a disease at once recognized as "Halo Spot." All 10 varieties in the test plats showed more or less spotting of the leaves, and the varieties showing the marked yellowing and stunting had abundant lesions on the stems both above and below the surface of the soil.

A few days later a number of fields of the Giant Stringless Greenpod variety, planted for a local cannery, were inspected and considerable infection found in every field planted. The loss in these fields was estimated at 10 to 25 per cent. The disease was also found in a number of gardens, in some cases causing severe damage, though generally infection was less severe on pole bean varieties. It was also found on both snap and lima beans throughout south Georgia.

The disease appeared in the fields again during the spring of 1930 but the season was comparatively dry and its development was quite different from that observed the previous year. The bean plats on the Experiment Station Farm were observed at frequent intervals and the relation of weather conditions to development and spread of the disease noted.

In one field an isolated 1/3 acre plat was planted April 22 with Giant Stringless Greenpod. No rain fell until May 20. On that date, just after the first shower, the field was searched carefully for infected seedlings. Five were found showing typical halo spots on the primary leaves. These were removed from the field. Light showers continued over a period of 10 days. On May 24th three infected areas were found.

[5]Figures in text refer to corresponding citations of literature on last page.

In each case only a few plants were involved but infection was quite abundant on these few plants.

Light showers fell again on June 4, 5, and 6. On June 12 a few new spots were found in each infected area. On June 13 to 17 light showers fell each day. A week later only a few new spots appeared. By June 26 the infected areas were still small, involving not more than five to six feet along the rows and had not spread to adjacent rows.

The plants were sprayed three times previous to this date with magnesium arsenate, which may have afforded some protection from this disease. No data are available as to the protective value of magnesium arsenate. However, weather conditions were doubtless the more important factor. Observations on inoculated plants indicate

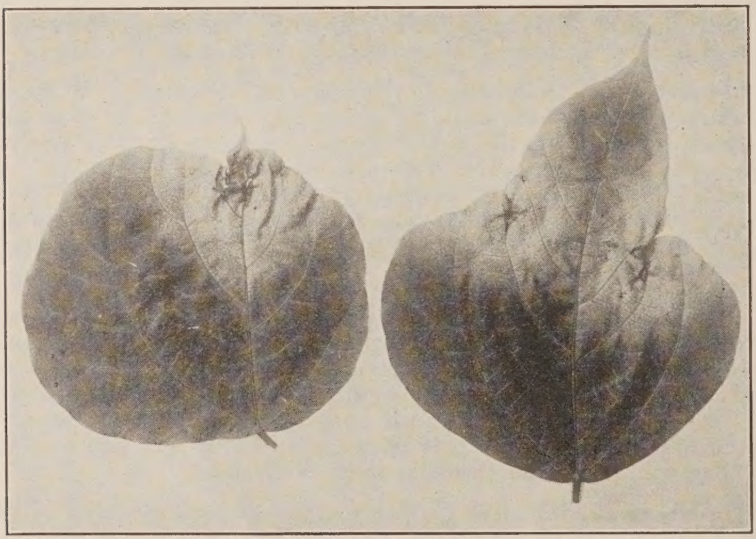

Fig. 1. "Halo Spot" on kudzu leaflets infected while very young, ⅔ natural size.

that the organism is very sensitive to moisture relations. Infection is very materially reduced and often entirely inhibited if the plants are allowed to dry to the wilting point even three or four days after inoculation.

SYMPTOMS OF DISEASE

In early stages of infection the leaves show very small (½ to 2 millimeters in diameter) spots bordered with a pale yellowish halo ½ to 2 centimeters (¼ to 1 inch) in diameter. Where several spots occur on a leaflet the entire leaflet may become chlorotic (pale yellow)

and more or less drawn and deformed. Later the dead areas enlarge and may split, causing a ragged appearance of the leaflet. In case of heavy infection the leaves die and drop from the plant in this stage.

On the leaf petioles and on stems of the plants larger, circular to elongated, dark brown spots occur, with or without a halo. Often a reddish-brown streak extends above and below the spot. Below the soil surface the spots are larger, often involving half the circumference of the stem. Apparently these spots are enlarged by Rhizoctonia and other soil fungi. In early stages the bacteria are abundant in these underground spots but later none can be found.

Where spots occur on the stem the leaves soon become chlorotic even when no spots occur on the leaves, and they finally drop off or die.

On the bean pods the spots are usually inconspicuous, small, water-soaked spots occasionally bordered with a very narrow halo; but during moist weather the water-soaked area enlarges rapidly and becomes very conspicuous. When green infected beans are picked and piled together over night the spots enlarge and become very conspicuous, even where no spots were noticeable when the beans were picked. The spots extend to the center of the pod and the seed are sometimes discolored.

Cause of the Disease

Microscopic examination of free-hand sections of the diseased spots shows an abundance of bacteria in the intercellular spaces of the host tissue. Often during damp weather, small droplets of bacteria in a viscid liquid are present on the diseased spots. If not washed off by rain, this exudate dries and is visible as a grayish white coating.

Isolations from Bean.—Bean leaves showing young spots were brought to the laboratory, the spots were cut out, washed with absolute alcohol to remove surface air bubbles, then 2 minutes in a 1–500 aqueous solution of mercuric chloride plus 5 drops of hydrochloric acid to each 100 cc. of solution, washed with sterile water, then, with sterile forceps crushed in a tube of melted nutrient agar, and dilution cultures made by transferring with a platinum loop to other tubes of melted agar and pouring into sterile petri dishes. In this way practically pure cultures of the causal organism were obtained; but in many cases yellow colonies, closely resembling those of *Bacterium phaseoli*, appeared. Numerous inoculation tests, however, showed the yellow organism to be non-pathogenic to beans.

Numerous isolations were also made from stem and pod lesions, using the same technique.

Late in the season cultures of *Bacterium phaseoli* were obtained from pod lesions. These cultures were used for inoculations and for physiological and morphological studies in comparison with the "Halo Spot" organism.

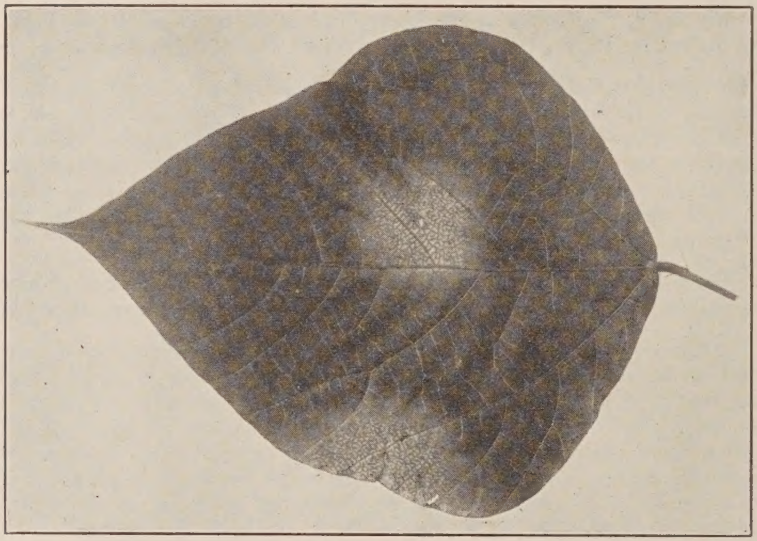

Fig. 2. Typical "Halo Spot" on mature leaflet of kudzu, $\frac{3}{4}$ natural size.

Isolations from Kudzu.—The superficial resemblance of the bean disease to the "Halo Spot" of kudzu, which is common wherever kudzu is grown throughout the State, led one to suspect that they might be produced by the same organism. Isolations were therefore made from the kudzu leaf spots using the same methods. The same types of bacterial colonies were obtained in the plates. The different types of colonies were isolated and later used for inoculation tests.

Isolations were made during late fall from small lesions on young stems of the kudzu vine, which yielded colonies of the halo blight organism.

From Lima Bean.—A bacterial spot was reported as being very destructive in some commercial plantings of lima beans near Tifton, Georgia. The latter part of June dealers refused to accept further shipments from one field. Some of the green pods were brought to the laboratory and isolations made by the method previously described and also by making dilution cultures directly from the viscid exudate which accumulated over the spots.

Isolations from Cowpea.—During spring and early summer of 1929 a bacterial spot was very destructive on young cowpea plants, causing defoliation and death of the plants. Since the organism (*B. vignae* Gardner & Kendrick) causing this disease has been said to attack lima beans, it seemed desirable to include it for comparison with the

"Halo Spot" organism. Several isolations were, therefore, made from young spots on cowpea leaves.

Cultures

Parallel cultures of the organisms isolated from the various hosts were run on a number of differential culture media. For this study three isolations from bean leaves, one from bean stem, two from kudzu leaves, one from kudzu stem, two from lima bean, three from cowpea, two of *B. phaseoli* from bean pods, and three of *B. vignae* from cowpea. All were run in duplicate or triplicate cultures on each medium used. All isolations used in the culture studies had been previously proven pathogenic to some of the host plants inoculated and the virulence of each culture was tested at intervals during the period of study. All cultures except gelatin were held at 25° C. The gelatin cultures were run at room temperature during winter 15° to 25° C.

The reactions of the forms from bean leaf, bean stem, kudzu leaf, kudzu stem, and from lima bean were essentially identical on all media, and may be characterized as follows:

Nutrient Agar.—On beef extract-peptone agar plates colonies are usually visible after 18 to 24 hours at 25° C. Surface colonies are bluish white to milk white, translucent by transmitted light, homogeneous, thin, flat; surface smooth and shiny. By the end of 6 days colonies on

Fig. 3. "Halo Spot" infection on seed leaves of bean seedling from infected seed. ⅔ natural size.

thinly sown plates are 2–3 mm. in diameter, edges more or less lobate. Submerged colonies are elliptical in outline, white, and usually less than 2 mm. in length. They usually come to the surface and develop similarly to surface colonies except for the more dense center. Bottom colonies are thin, bluish-white, with smooth edges.

In Stab cultures in tubes of nutrient agar the growth is echinate along stab, slightly raised and convex on surface with an opalescent white membrane over the entire surface.

On nutrient agar slants growth is scant, echinulate, with an opalescent white membrane over entire surface of the agar.

In shake cultures the colonies are small, bead-like and confined to upper 2 millimeters.

Bouillon.—Beef extract-peptone broth is clouded after 24 hours and a white flocculent membrane is formed on the surface after 2 to 3 days, which breaks and settles to bottom on slight shaking. The broth does not clear on standing, and sediment remains flocculent.

Fermentation of Carbohydrates.—The carbohydrate to be studied was added to beef extract-peptone broth and agar to make 1 per cent solutions, tubed and sterilized.

Dextrose.—On beef extract-peptone broth plus 1 per cent dextrose growth was similar to that on broth alone, but more copious. After two days a heavy white membrane had formed on the surface which, on slight shaking, broke and settled as flocculent precipitate. In fermentation tubes the open arm was densely cloudy after 48 hours. The closed arm clouded more slowly but was slightly clouded throughout by the fourth day. No gas was formed.

In dextrose broth plus brom cresol purple as indicator (initial reaction about pH = 6.6) no change in reaction was visible until end of second day when the top of the liquid about 5 mm. deep had changed to the full yellow color of the indicator. After this the reaction changed rapidly both at top and bottom of the liquid, and by the end of four days the reaction was about pH = 5.4 where it remained fairly constant during three weeks.

Saccharose.—In broth plus 1 per cent saccharose growth and reaction changes were similar to those in dextrose broth. No gas was formed.

Lactose.—In broth plus 1 per cent lactose growth was poor, little if any better than in broth alone. In fermentation tubes the closed arm of the tube was never perceptibly clouded. No gas was formed. In tubes with brom cresol purple added the cultures all became gradually more alkaline.

Maltose.—In broth plus 1 per cent maltose the growth and reaction changes were similar to those of lactose broth. No gas was produced in fermentation tubes. Apparently, no acid was formed since the cultures with indicator added all became more alkaline.

Glycerin.—In broth plus 1 per cent glycerin growth was more abundant than with any of the sugars. In fermentation tubes the medium became densely cloudy in both the open and the closed arm.

Fig. 4. Bean variety plats showing effect of "Halo Spot" disease on Bountiful variety, Giant Stringless Greenpod on either side, Wardwell's Kidney Wax in the distance and a few plants of Refugee 1000–1 in the foreground.

No gas was formed. In glycerin broth plus brom cresol purple more acid was formed than with either dextrose or saccharose, the final reaction being near pH = 5.0.

Digestion of Starch.—With stroke cultures on plates of nutrient agar plus 2 per cent corn starch slight clearing of the agar immediately under the bacterial growth could be seen after 8 days. The disappearance of the starch was verified by examination under the microscope and by the iodine test. In tubes a slight clearing could be seen in the upper part of the agar but the cleared area was not more than 2 to 3 millimeters deep at the end of 6 weeks.

Skim Milk.—In tubes of skim milk there was no curdling or digestion of the milk up to the end of the test, 21 days, and very little visible growth. In tubes of milk plus brom cresol purple the medium became slightly more alkaline at the top after 5 days and alkaline throughout after 12 days.

Nutrient Gelatin.—Stab cultures in tubes of beef extract-peptone gelatin showed good growth on the surface after five days, but no or very slight liquefaction at the end of 21 days. After six weeks the upper half inch of the gelatin was liquefied to a concave floor. Liquefaction had advanced little further when the cultures were thrown out at the end of three months.

Dunham's Peptone Solution.—In a 1 per cent solution of peptone growth was similar to that in beef extract-peptone broth with slightly heavier growth as indicated by sediment.

Fermi's Solution.—Good growth was obtained in Fermi's solution. It was distinctly clouded after 48 hours with a greenish opalescent hue. After 7 days there was a heavy precipitate at the bottom with a dense membrane at the surface, breaking flocculent on shaking.

Ammonia Production.—Ammonia was produced in all media containing peptone.

Indol Production.—In tubes of Dunham's peptone solution no indol was found when tested after three days and again after three weeks growth. The cultures were tested both by the sulphuric acid-sodium nitrite and by the p-dimethyl amidobenzaldehyde-hydrochloric acid tests with negative results.

Nitrate Reduction.—In beef extract-peptone broth plus 1 per cent potassium nitrate no nitrites were found when tested on the fourth and tenth days.

The principal differences in the physiological characteristics of the three organisms studied may be seen in Table 1.

Table 1

Characteristic Differences in the Culture Reactions of the Three Species

	B. medicaginis var. *phaseolicola*	*B. phaseoli*	*B. vignae*
Color of agar colonies	Bluish white	Yellow	Grayish white
Acid production	+From dextrose, saccharose, and glycerin.	None	+From dextrose and saccharose. None from glycerin.
Nitrate reduction	None	None	Slight at end of 10 days.
Digestion of gelatin	Very slow	Rapid	Slow
Digestion of milk	None, no curdling	Rapid and complete. No curdling.	None or very slight. No curdling.
Fermi's Solution	Heavy growth, greenish opalescent color produced.	Growth none or very slight. No change in color.	Heavy growth. No greenish color.

Inoculations

Inoculations were made on a number of leguminous plants, including beans (Bountiful and Giant Stringless Greenpod varieties), lima bean, kudzu, cowpea, velvet bean, alfalfa, and soybean, using the halo organism. The bacteria isolated from bean, lima bean, and kudzu were used under comparable conditions. For comparison, inoculations were made with *Bacterium phaseoli*, isolated from bean pods, and with *B. vignae* Gardner and Kendrick isolated from cowpea.

The results are shown in Table 2.

Stomatol infections with *B. Medicaginis var. phaseolicola* were obtained on string bean, lima bean, kudzu (*Pueraria thumbergiana*). None were obtained on soybean or alfalfa, and doubtful on cowpea and Florida velvet bean (*Stizolobium deeringianum Bort.*). On the latter black spots developed consistently but attempts to reisolate the bacteria always gave negative results.

In most cases the bacteria from a young agar culture were suspended in sterile water and sprayed with an atomizer onto the leaves of pot grown seedlings in the greenhouse. Usually the plants were covered with bell glasses for a preliminary period of 24 hours, sprayed with the bacterial suspension, and then covered again for another 24 hour period. In a few cases, during cloudy damp days, the plants were inoculated without covering.

On bean and kudzu plants sprayed and kept under bell glasses for 24 hours the first noticeable symptom of the disease was more or less circular pale spots on the leaves, usually evident on the fourth day. These pale, chlorotic areas continued to enlarge and become more distinct until about the eighth day. By this time a small (½ to 1 millimeter in diameter) dead spot could be seen at the center. Under very humid conditions the infected center appeared water-soaked for perhaps one or two days, then changed to light brown in color. The dead area then gradually enlarged, sometimes to a more or less angular area 5 millimeters in diameter. The halo around the dead area was usually one to two centimeters (roughly ½ to 1 inch) in diameter. When infection was very abundant on a leaf the whole leaf became chlorotic and the individual spots were rather inconspicuous. Very young leaves often showed stunting and distortion, especially where the infected area included a larger vein. When the plants were kept under very moist conditions during the entire incubation period the infected spots showed as water soaked areas on the under side of the leaves.

The bean plants of the Bountiful variety often showed stem lesions at or near the soil line. Such plants usually became chlorotic throughout and noticeably stunted.

On plants sprayed with the bacterial suspension without covering infection was never so abundant and the incubation period was longer,

usually about seven days. This probably approaches the incubation period under field conditions.

On lima beans the spots were larger, 1 to 3 millimeters in diameter, with a very narrow chlorotic border. On the Fordhook variety a distinct halo appeared, similar to that on string beans.

Inoculations were also made on bean and lima bean with cultures from bean and kudzu by pricking the bacteria into a stem node with a sterile needle, then covering the plants with a bell jar or wrapping the inoculated portion of the stem with wet absorbent cotton. Either method usually gave nearly 100 per cent infection. After five to ten

Fig. 5. Bean variety plats showing effect of "Halo Spot" disease on Wardwell's Kidney Wax variety, four rows. Rust Proof Wax at left, one row of Giant Stringless Greenpod at right.

days a small water-soaked area could be seen about the needle punctures and a few days later bacterial exudate was present over this spot, reddish-brown streaks extended along the stem mostly above the point of inoculation, and the plants began to show chlorosis and stunting. Soon after the lower leaves of the string bean began to wilt and flag or drop off, and by the end of a month some of the plants were dead above the point of inoculation. At this time some of the living plants had droplets of bacterial exudate at several nodes, coming from stipule scars. No spotting of the leaves occurred.

The lima beans showed similar symptoms in a milder form. All showed some stunting and chlorosis but only a small percentage of the plants were killed outright.

One month after inoculation isolations were made from nodes and internodes of bean plants above the point of inoculation and from the

internode about two inches below the point of inoculation, also from the pulvinus and petiole of chlorotic leaves. The bacteria were very abundant in the first internode above the point of inoculation, and gradually decreased in the succeeding internodes toward the top of the plant. They were much less abundant below than above the inoculation point. They were much more abundant in the nodes than in the internodes. Sections from petioles of chlorotic leaves gave few colonies but the pulvinus from the same leaves gave very abundant growth of bacteria.

This distribution of the bacteria was confirmed by microscopic examination of stained sections of petiole and pulvinus. In the petiole sections the bacteria were very few and appeared to be confined to the ducts and a few adjoining wood parenchyma cells. In the pulvinus many of the ducts were packed with bacteria and quite often some of the surrounding parenchyma cells were broken down and a cavity formed which was completely packed with bacteria. These cavities were all confined to the woody cylinder. The bacteria were never found in the cortical parenchyma or anywhere outside the central cylinder. Apparently the action producing the cavities was entirely mechanical. One might naturally expect this force to split the tissues to the surface. However, such splitting has never been observed in field plants or in plants artificially inoculated. The only places where the bacteria have been observed to reach the surface of such so-called[1] &[2] systematically infected plants are the primary stem lesion, stipule scars, and leaf scars. Apparently the bacteria are merely carried along the water ducts by the water movement, and from this pass occasionally through connecting pits into the adjoining parenchyma cells where multiplication occurs for some time.

The chlorosis or yellowing of such internally infected plants is doubtless due to some enzyme or toxic substance produced by the bacteria, which in some way destroys the green coloring matter, chlorophyl, in the affected area.

Several attempts to isolate the bacteria from the upper nodes o chlorotic field plants have in many cases given negative results. Cultures of stem tissue from chlorotic kudzu shoots, growing from cankered vines have always given negative results. In fact the bacteria appear to be confined to the immediate neighborhood of cankers in the kudzu stem, while shoots arising some distance above old overwintered cankers may be chlorotic.

Table 2

Source of Culture	Plants inoculated	Number of plants	Method of inoculation	First symptoms of infection	Number infected	Remarks
Bean leaf cultures No. 7, No. 8, and No. 108.	Bean	31	Atomizer	4–7 days	31	Typical small spots with wide halo on leaves.
	Lima bean	19	Atomizer	None	7	
	Kudzu	4	Atomizer	4 days	4	Typical small spots with wide halo.
	Cowpea	15	Atomizer	None	0	
	Alfalfa	50	Atomizer	None	0	
	Velvet bean	3	Atomizer	2 days	3	Black spots on young leaves.
	Bean	15	Needle puncture in stems	5–10 days	14	Water-soaked area about puncture, followed by reddish-brown streaks with yellowing stunting, and final death of plants.
	Lima bean	5	Needle puncture in stems	10 days	5	Same but symptoms less marked than in string bean.
Bean stem culture No. 2.	Bean	8	Atomizer	5 days	8	Typical halo spot on leaves.
	Lima bean	9	Atomizer	None	0	
Kudzu leaf cultures No. 13 and 113.	Bean	30	Atomizer	4–6 days	30	Typical small spots with wide halo.
	Kudzu	6	Atomizer	4 days	6	Typical small spots with wide halo.
	Lima bean	10	Atomizer	None	0	
	Velvet bean	4	Atomizer	2 days	4	Black spots on young leaves.
	Alfalfa	50	Atomizer	None	0	
Kudzu stem culture No. 11.	Bean	1	Atomizer	5 days	1	Typical halo spots on leaves.
	Lima bean	4	Atomizer	5 days	4	Red-brown spots on seed leaves, on true leaves small (2–3 mm.) spots with very narrow halo.
	Cowpea	3	Atomizer	5 days	3	Few very small red-brown spots, no halo.

Source of Culture	Plants inoculated	Number of plants	Method of inoculation	First symptoms of infection	Number infected	Remarks
Lima bean pods culture No. 1 and No. 2.	Bean	42	Atomizer	4–7 days	29	Typical small spots with wide halo on leaves.
	Kudzu	13	Atomizer	None	1	Typical halo spots on several leaves.
	Lima Bean	49	Atomizer	None	4	Nearly all inoculations on Jackson Wonder, which appears to be immune to stomatal infection.
	Velvet bean	10	Atomizer	2 days	6	Black spots on young leaves.
	Cowpea	77	Atomizer	None	0	
B. phaseoli from bean pod. Cultures No. 9, 110, and 119.	Bean	55	Atomizer	4–9 days	55	Spots numerous, large (up to 5 mm.) angular; little or no halo.
	Kudzu	16	Atomizer	4 days	4	Spots few, no halo.
	Lima bean	30	Atomizer	4–9 days	30	Similar to bean, spots slightly smaller.
	Velvet bean	4	Atomizer	None	0	
	Cowpea	31	Atomizer	None	0	
B. vignae from cowpea. Cultures No. 2, 3, and 102.	Bean	7	Atomizer	None	0	
	Kudzu	8	Atomizer	None	0	
	Lima Bean	2	Atomizer	5 days	2	Large reddish-brown spots on seed leaves, small pale spots with brown borders on true leaves.
	Cowpea	45	Atomizer	4–6 days	45	Pale spots (2–5 mm.) with reddish-brown borders on leaves, reddish-brown spots and streaks on stems and leaf petioles.

Varietal Susceptibility

During the spring of 1930 ten varieties of bush type string beans were grown in the horticultural test plats on the Experiment Station Farm. They were planted in plats of 4 half-rows to each variety. Each variety was thus brought into close contact with three other varieties in the field. Weather conditions appeared to be almost ideal for dissemination and development of the disease. Under these conditions the amount of infection and the injury occurring naturally on each variety would seem to furnish a reliable standard of comparison as to susceptibility of the variety.

It may be noted in Table 3 that in three varieties every plant showed more or less leaf infection. In these varieties stem infection, accompanied by loss of green color, stunted growth, and final death of the plants was common. A few days after these notes were made the remaining plants of the Bountiful variety died. Only a few pods of this variety matured.

At the other extreme we find the Valentine and Refugee 1000 to 1 varieties with only an occasional leaf spot and no serious reduction of yield. However, the green pods of all varieties showed more or less spotting when picked and held over night in baskets in a moist atmosphere.

No lima beans were grown near these plats and no direct comparison of varieties of this group has been made under field conditions. However, both Henderson Bush Lima and the Fordhook varieties showed heavy damage to both foliage and pods in south Georgia during 1929. In inoculation tests the Fordhook appeared to be very susceptible. The Henderson Bush Lima was only slightly less susceptible, while Jackson Wonder appeared to be entirely immune to stomatal infection. Needle-prick inoculations on the latter gave a high percentage of infection with stunted growth, leaf casting, and in some cases, death of the plant above the point of inoculation.

Table 3

Comparative Susceptibility of 10 Commercial Varieties of Bunch Beans, from Notes Made June 4, 1929.

Rank	Variety	Infection per cent	Plants killed per cent	Remarks
1	Bountiful	100	90	Remainder yellow and stunted.
2	Wardwell's Kidney Wax	100	75	Remainder yellow and stunted.
3	Stringless Greenpod	100	60	25 per cent yellow and stunted.
4	Giant Stringless Greenpod	50	1	10 per cent yellow and stunted.
5	Tennessee Greenpod	25	None	2 per cent yellow and stunted.
6	Rust Proof Wax	10	None	None yellow or stunted.
7	Excelsior Wax	5	None	None yellow or stunted.
8	1000 to 1 Refugee	2	None	None yellow or stunted.
9	Extra Early Valentine	1	None	None yellow or stunted.
10	Black Valentine	Slight	None	None yellow or stunted.

Source of Infection and Means of Dissemination

After finding that the disease could be transferred readily from kudzu to beans, the kudzu vines of the region were examined for the "Halo Spot" disease. In nearly all cases heavy infection was found on kudzu leaves and cankers were frequently found on the stems. It seemed, therefore, that the organism might live over winter in these stem cankers and become a very important source of infection for beans, and an investigation of this point was planned.

The bark of young kudzu stems is light green in color and the cankers may be seen as darker green, somewhat water-soaked spots which soon change to a dirty brown color. As the vines age during the fall months the color of the bark changes to a dark grayish-brown almost identical with the color of the cankers. The cankers are therefore very inconspicuous and difficult to find and identify with certainty during the winter. The infection often extends only through the bark and causes no noticeable shrinking or distortion of the tissues. Occasionally the xylem is attacked and killed entirely to the pith; and growth of the stem causes some cracking and overgrowth about the canker.

All of the younger growth of kudzu vines winter-kills in this region, leaving alive only the larger thoroughly hardened stems and those protected by fallen leaves.

During the spring of 1930 the first new growth of kudzu in the area under observation appeared about April 10th. On April 18th rain fell almost continuously throughout the day. Six days later a few "Halo" spots were found on leaves, all grouped about three centers. At two of these centers an old stem canker was found directly beneath some diseased leaves. A week later several more infection centers were found and examination of the vines beneath resulted in finding seven more cankers. In the same way two more were found on May 5.

In some cankers the infected area did not extend entirely through the bark but in others the xylem tissue was involved to the pith and included an area two to four inches long and half the circumference of the stem. After the outer bark was removed the tissue toward the center of the canker was dead and brown. Around the borders of the dead area the tissues were water-soaked and pockets of bacterial growth were sometimes visible. In a few cases bacterial exudate was visible on the surface.

Sections of the stems containing the cankers were removed, surface sterilized, and the outer bark removed with a flamed scalpel. Canker tissue was then removed with another sterile scalpel and crushed in a tube of sterile agar from which dilution cultures were then plated.

Nine of the 11 cankers gave abundant colonies of *B. medicaginis* var. *phaseolicola*. Some of these were isolated and used in successful inoculations of bean plants.

Cultures of the surrounding stem tissue above and below the cankers remained sterile in every case. Apparently the bacteria do not pass along the ducts as they sometimes do in the bean stem.

On May 5 several chlorotic sprouts were found growing out from cankered stems. The growth of these sprouts appeared to be stunted, the leaves were pale yellowish-green, about one-third normal size, often crumpled and distorted, and dead areas occurred usually around the edges of the leaflets. Four such chlorotic sprouts were examined for the presence of bacteria with negative results. Cultures were made from the leaves including the dead areas, the pulvinus, stem nodes and internodes, and the old stems above and below the cankers as well as the canker tissues. No bacterial colonies developed in any of the cultures except those from the canker tissue. None were obtained from the new growth, showing conclusively that the chlorosis of the new growth was caused by action of the bacteria in the old cankered tissue. Probably some toxic substance produced in the old cankers was carried along the conducting tissue and caused the chlorosis and stunting of new growth.

This same effect is easily demonstrated in the chlorotic halo about the spots on kudzu leaves. Here the halo is sometimes two inches in diameter. Repeated attempts to isolate the bacteria from the outer portions of this chlorotic area have resulted in failure even when veins crossing the dead spot were included.

Continued, almost daily, observation of this area of kudzu indicates that infection spread from these centers, found early in the spring, entirely by spattering of rain drops. After each rainy period each infected area enlarged until the entire field was covered by the middle of June. During periods of two to four weeks during which no rain fell no new infections were found.

These observations indicate that the stem cankers in living stems are the principal source of spring infection. It seems doubtful that the dead stems and leaves contain living bacteria. At least no infections have been found that could be attributed to this source, though the leaves and young stems were heavily infected during the previous fall and were allowed to lie on the ground throughout the winter and spring.

Evidently diseased kudzu may be an important source of infection for adjoining bean fields. In fact heavy windstorms might spread the disease for some distance but during an ordinary season it is doubtful whether the bacteria will be spread more than a few yards from the vines.

Observations in bean fields indicate that diseased seeds are the most important source of infection. In several fields the disease has been found localized in more or less circular areas, spreading after each rain just as described for kudzu.

Control

The organism producing "Halo Spot" is borne on the surface of the seed and also internally in, usually, small inconspicuous lesions. It possibly lives over winter in dead vines and litter in the field; though on this point we have no direct experimental evidence.

Our results show conclusively that it does live over winter in old cankers on living kudzu vines. Observations indicate, however, that the bacteria are spread from plant to plant principally by spattering of rain drops, and that the kudzu is not an important source of infection under ordinary conditions unless in close proximity to the bean field.

The principal source of infection appears to be the seed beans. The bacteria on the surface of the seed beans may be destroyed by disinfecting the surface of the seed before planting but such treatment will not kill those inside the seed coat.

Last year tests were started in an effort to determine the value of seed treatment. Ten pounds of seed of the Bountiful variety, from a lot previously found to carry infection, were divided into three equal lots. One lot was soaked 20 minutes in a mercuric chloride solution, 1 part mercuric chloride to 1000 parts of water; the second soaked 30 minutes in Semesan solution, 1 part Semesan to 400 parts water; and the third

lot left untreated. All were planted at once, without drying on June 8, 1929.

There was no apparent injury from either of the treatments. A good stand was obtained in each plat.

On July 5 the seedlings were carefully examined for disease. No diseased seedlings were found in either of the plats planted with treated seed; and only three were found in the untreated plat.

While the results might be interpreted as indicating control by seed treatment, they are not sufficiently conclusive to justify recommendation of seed treatment.

The grower must therefore make every effort to obtain seed free from disease. Halo Spot has not yet been found in some seed-growing districts of the West but it is prevalent in other districts. Apparently no district is free from Halo Spot every year except in the irrigated regions of the Southwest. The occurrence and destructiveness of the disease varies from year to year, dependent upon weather conditions. The only safe course, therefore, is to insist upon seed certified as disease-free by responsible authorities.

Since this disease does not spread during dry weather there is a promising possibility of growing disease-free seed during late summer, especially in the Piedmont section of Georgia.

As shown in Table 3 there is great variation among the popular string bean varieties as regards their susceptibility to this disease. While other considerations, such as resistance to other diseases, yield, and market demands, must be considered by the bean grower, varieties that are very susceptible to halo spot should not be planted on a large scale in this State.

The important control measures to be recommended at this time are:

1. Select the most resistant variety that is suitable to the market demands.

2. Plant disease-free seed when such seed are obtainable.

3. Avoid planting beans in close proximity to kudzu.

The latter is of special importance because the disease may spread from bean to the kudzu, if it is not already diseased, and the kudzu will then remain as a perpetual source of infection.

SUMMARY AND CONCLUSIONS

The results confirm the recently published conclusion of Miss Hedges that the "Halo Spot" of beans and a similar disease of kudzu are produced by the same organism, *B. medicaginis* var. *phaseolicola* Burk.

On kudzu the bacteria overwinter in cankers on the living vines; and may be an important source of infection for nearby bean fields.

Beans should not be planted near kudzu vines.

The bacteria are also carried by bean seed from infected fields.

Infected seed were responsible for most of the outbreaks of the disease observed in Georgia.

Seed beans certified free from disease should be used for planting when such certified seed are obtainable.

Bean varieties vary greatly as to susceptibility to the disease. Refugee 1000–1 is very resistant while Bountiful is extremely susceptible, and all gradations between these two extremes are found among the popular canning varieties.

The damage produced by the disease depends upon weather conditions. During years of frequent rainfall and high humidity during the bean-growing season severe damage may be expected.

Observations indicate that disease free seed may be produced in the Piedmont section of Georgia by planting during June or July.

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